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FOREST INSECT INVESTIGATIONS

MEMORANDUM ON NUTRITIONAL STUDIES OF WESTERN PINE BEETLE LARVAE

by

GEORGE R. STRUBLE
Bureau of Entomology

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Introduction

During the three months from January 1 to April 1, 1930, some intensive studies were carried on at the California Forest Experiment Station to determine, if possible, the nutritional requirements of the larvae of the western pine beetle. This problem was taken up as the result of investigations into the life history of this insect. It is a puzzling question how nutrition is obtained from yellow pine bark, especially the outer bark, which is so low in food materials. Yet we know that the larvae are able to complete their development within four to six weeks following the time the eggs are laid.

This problem may not appear at the outset to be of much practical value, yet it is felt that a study of this kind will contribute considerable to our knowledge of this important economic forest insect, and may in the end prove of great value as investigations are carried out on the host tree itself.

Most of the results of this study were negative, but this was to be expected, since all variable factors concerned with the environment of this insect cannot be known except through much experimentation.

Methods

Supply of Living Material

All experiments carried on in this study were begun with the egg stage of the insect. The eggs were obtained by rearing the insects under artificial conditions. During the fall of 1929 a quantity of bark containing winter broods of D.b. was brought into the laboratory and placed in cold storage. At the same time several green yellow pine logs were also placed in cold storage. As needed, a log was placed in a cage with brood bark at room temperature. Within a period of usually two weeks sufficient attacks had been made and eggs laid by the new adult beetles which had emerged from the brood bark to permit the carrying on of experiments on nutrition. When the supply of eggs ran low, another log and more brood bark from cold storage were placed at room temperature. In this way a constant supply of fresh eggs could be maintained.

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Rearing Equipment

The fact that very few experiments have been carried on with insects similar in nature to D.b. larvae made it rather difficult to know what equipment would be most suitable for this work. Consequently it was necessary to use several different types of equipment and eliminate that which proved unsatisfactory. Glass tumblers were first tried, but proved very unsatisfactory both on account of the difficulty in handling and the inability to keep the culture media free from outside contamination. Petri dishes were found to be more suitable than tumblers, chiefly because of the fact that they could be easily sterilized. Several experiments were carried out using them, but it was found that they too could not be kept sterile. The introduction of eggs or larvae permitted also the entrance of fungus spores from the air, and by the end of two or three weeks the media would be completely overrun with fungus development. This necessitated the use of a different type of equipment. Crystallizing dishes (40 mm.depth by 60 mm. width) were next used, chiefly on account of their smaller diameter compared with Petri dishes and because they were thought less apt to become contaminated. They too, however, were very unsuccessful in keeping out fungus spores at the time D.b. eggs were put in.

Test tubes were tried out with a certain degree of success, but finally eliminated on account of having too small an opening and too great a depth. Shell vials were next tried, and up to the present have been the most successful of any rearing equipment. The one-inch diameter by four-inch depth is the most convenient size.

Media

The difficulty of establishing an environment which would even approach the conditions to which a D.b. larva is naturally accustomed also led to much experimentation. Assuming that a medium composed of pure cellulose would furnish surrounding similar to inner yellow pine bark, a number of cellulose preparations were tried. Cellulo filter pulp (almost pure cellulose made from spruce pulp) was tried in a number of experiments both with and without the addition of nutrients. Trial was also made of other media, viz., filter paper, agar-agar, elder pith, ground inner yellow pine bark, ground outer bark and rice.

Nutrients

As has already been pointed out, very little--in fact, nothing --is known concerning the nutritional requirements of D.b. larvae. Consequently the only guide to a nutritional study is furnished by the literature dealing with nutritional work on other unrelated species of insects, notably Drosophila, flesh-fly larvae, and termites. Baumberger[1] and Cleveland[2] have contributed the greatest amount of information on this subject. Uvarov has also aided to a great extent by compiling all the known facts concerning nutritional studies with insects, as well as by giving a complete bibliography on the subject. An analysis of the sugar content of inner yellow pine bark by Jeffrey[4] has also been of great assistance in working up nutrient solutions. In fact, the sugar content is probably the most important source of nourishment.

The fact cannot be overlooked that certain microorganisms intimately associated with most of the wood-boring insects may also be important in this species. A yeast has been found definitely associated with the adult and larva of D.b., and experimentation may prove it to be essential to the development of D.brevicomis.

With the amount of information given, a number of solutions were prepared, using the following substances in varying proportions: sugars--maltose, levulose, sucrose and dextrose; nitrogen, supplied by either bacto-peptone or ammonium tartrate; salts--magnesium sulphate and potassium phosphate. Sterile tap water and a hot-water extract of inner yellow pine bark were added to certain media instead of nutrients and used as checks against them.

The following solutions of sugars were used, and will hereafter be referred to by the numbers or letters given them:

A. Dextrose 16.5 gms.; sucrose 16.5 gms.; ammonium tartrate 3.3 gms.; potassium phosphate 0.165 g.; magnesium sulphate 0.165 g.; water 200 cc.; agar-agar 4 gms.; pH 6.1.

F. Maltose 4 gms.; dextrose 4 gms.; sucrose 4 gms.; potassium phosphate 0.165 g.; magnesium sulphate 0.165 g.; agar-agar 4 gms.; cellulose 3 gms.; water 200 cc.

I. Dextrose 2 gms.; sucrose 2 gms.; maltose 2 gms.; peptone 1 gm.; magnesium sulphate 0.082 gm; potassium phosphate 0.082 g.; water 150 cc.; pH 5.4.

#8. Same as A with exception that no agar used, but instead cellulo pulp used as medium.

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[1]Baumberger, J.P. A nutritional study of insects. Jour.Exp.Zool. 28:1-81; 1919

[2]Cleveland, L.R. Am.Jour.Hygient; 3:444-461; 1923
Biol.Bul.Woods Hole,Mass. 46:178-227; 1924

[3]Uvarov, B.P. Insect nutrition and metabolism; a summary of the literature. Trans.Ent.Soc.London; 1928

[4]Jeffrey, R.N. The concentration of certain sugars in the bark of the western yellow pine as related to western pine beetle attraction;ms;1930

#10. Dextrose 5 gms.; sucrose 7.6 gms.; maltose 7.8 gms.; potassium phosphate 0.165g.; magnesium sulphate 0.165 g.; ammonium tartrate 2 gms.; water 200 cc.; pH 6.7.

#13. Dextrose 5 gms.; sucrose 7.6 gms.; levulose 7.8 gms.; peptone 2 gms.; potassium phosphate 0.165 gms.; magnesium sulphate 0.165 gms.; water 200 cc.; citric acid to pH 4.4.

18. Dextrose 1 gm.; sucrose 1.8 gms.; levulose 2.6 gms.; potassium phosphate 0.165 g.; magnesium sulphate 0.165 g.; peptone 2 gms.; water 200 cc.; pH 4.7.

#22. Dextrose 3.54 gms.; sucrose 7.63 gms.; levulose 3.58 gms.; peptone 2 gms.; potassium phosphate 0.165 g.; magnesium sulphate 0.165 g.; water 200 cc.; pH 4.9.

Technic

Sterilization of equipment and media. In a study of this kind it is of the greatest importance that methods of handling material and of keeping out outside contamination be developed. The sterilization technic employed in this study was done by means of heat supplied by an electric oven and a steam autoclave.

1. Petri dishes. Before introducing media of any kind, the petri dishes were thoroughly dried and sterilized in an electric oven at 250° F. for at least half an hour. The technic used for sterilizing media in petri dishes varied according to the type of medium used. If a solid, such as cellulose pulp saturated with nutrients or water, it was placed in the petri dish and sterilized in the autoclave for 20 minutes at 240° F. If a liquid, it was sterilized by boiling and then poured into sterlized petri dishes. This technic was also used for crystallizing dishes.

2. Test tubes and shell vials. The sterilization of test tubes and shell vials was accomplished by placing them in the electric oven at 250° F. for not less than 45 minutes. When the media were placed in the vials they were again sterilized at 200° F. in the electric oven for variable lengths of time, from 1 to 8 hours. This also varied the moisture content of the media.

Handling of living material. The living material was obtained by rearing in fresh green logs. When sufficient eggs had been laid the outer bark was removed by means of a hand axe, so that the inner bark, containing the egg galleries, could be stripped off with a pocket knife without very much loss or injury to the eggs. All eggs were carefully removed from the galleries by first stripping the bark away from the egg niches by means of forceps. The eggs were then carefully picked up with a moist camelshair brush and placed in a moist chamber until ready to be planted on media.

1. Transfers to petri dishes. As soon as the media were ready, the eggs were immersed in a .1 per cent solution of mercury bichloride to sterilize the outside, rinsed in sterile water, and transferred to the media by means of a sterilized camelshair brush. (Experimentation showed that eggs immersed in this way were just as viable as without the treatment.) At the time the transfers were made the petri dishes were opened barely enough to insert brush and egg, and were closed immediately after each egg was planted. Technic as careful as this did not prevent the introduction of fungus spores. This method was also used for crystallizing dishes.

2. Transfers to shell vials. With this type of equipment a special method was devised whereby the eggs were placed in niches between glass and medium. The niches were made by means of a sterilized (flamed) dissecting needle, ten being made in each vial. While the niches were being made the mough of the vial was kept flamed and inverted, thus preventing the introduction of spores from the air. Eggs were then introduced, one to each niche, by means of a sterilized camelshair brush. (The brush was sterilized with mercury bichloride and the handle flamed before each transfer.) The vials were then stoppered with cotton plugs or cork stoppers, depending on the desired moisture content of the medium. This method was very much of an improvement over the petri dishes and the other methods tried, for three reasons: (1) on account of better sterilization, as it was found that absolute freedom from outside contamination could be maintained; (2) because the niches made the environment of the newly-hatched larva more nearly natural; and (3) because observations could be made without the risk of contaminating the media.

Tabulation of Data

The data recorded herein are the results of experiments carried on with the two best types of rearing equipment, viz., petri dishes and shell vials. The results obtained from the use of other types of equipment are too inconclusive to consider.

Petri Dishes

The data for this type of equipment are recorded by Table I in groups according to the nutrient solution used. The latter are represanted by numbers and letters, the details of which have already been given.

Table I

Rearing in Petri Dishes

Medium	pH	Date Begun	Date Ended	Total Days	Average
Cellulo-filter pulp	7.1	Jan. 3	Jan. 22	19	
		" 3	" 16	13	
		" 3	" 22	19	
		" 3	" 22	19	
		" 3	" 16	13	
		" 10	" 22	12	15.8
A	6.1	" 7	" 20	13	
		" 7	" 20	13	
		" 7	" 16	9	
		" 7	" 22	15	12.0
		" 7	" 16	9	
F		" 8	" 20	12	
		" 8	" 20	12	
		" 8	" 16	8	10.6
8	6.1	" 6	" 22	16	
		" 6	" 20	14	
		" 6	" 16	10	
		" 6	" 20	14	13.5
I	5.4	" 10	" 22	12	
		" 10	" 22	12	12.0
10	6.7	" 20	Feb. 3	14	
		" 20	" 3	14	
		" 20	" 5	16	14.6
13	4.4	" 21	" 3	13	
		" 21	" 3	13	
		" 21	" 3	13	
		" 21	" 3	13	
		" 22	" 5	14	13.2
18	4.7	" 22	" 5	14	
		" 22	" 5	14	
		" 22	" 5	14	
		" 24	" 5	12	13.5
22	4.9	" 24	" 3	10	
		" 25	" 5	11	
		" 25	" 5	11	10.6

This table does not show the exact period which the larvae survived, but it was noted that the eggs in practically every instance had hatched within one to five days after transferring to petri dishes.

The most striking feature of these experiments is that the longest period of survival was attained by the larvae which were living on cellulose saturated with water and therefore entirely lacking in nutriment. This may seem rather strange on first consideration, but it is not surprising when in practically every case where nutrient solutions were used the media were very heavily contaminated with fungus mycelium and spores within a week to ten days after the eggs were planted. This condition undoubtedly affected the development of the larvae.

Very little can be said on the significance of any one nutrient solution. The fact that there was a longer period of survival on some of the nutrients evidently indicates that there was less fungus contamination.

Shell Vials

As this type of equipment was found to be much more satisfactory than petri dishes from the standpoint of contamination, a definite procedure was followed, using cellulo filter pulp as a basis for Solution #18, which was chosen as nearest to the natural sugar content of a yellow pine. Not knowing the optimum amount of moisture necessary for the development of D.b. larvae, the moisture content of each vial was varied by drying for different periods in an electric drying oven; 1,2, 4,6 and 8 hours were arbitrarily chosen. The pH was varied also with each drying time, using pH 7,6,5,4 and 3, so that for a given drying time there were five pH variables. In all, 30 vials were used, as follows: 25 with nutrient solutions and variables according to pH and drying time, and 5 checks using cellulo pulp saturated with water and dried for the periods of time mentioned. The results are summed up in Table II. Ten eggs were placed in each vial.

Table II

Nutrient	Vial No.	pH	Drying Time; Hours	Date Eggs In	Date First Larvae	No. of First Larvae	Date of Last Death	Days	Average
Water	1	6.8	1	2/10	2/11	5	2/20	9	
	2		2	2/10	2/13	2	2/20	7	
	3		4	2/11	2/14	1	2/27	13	
	4		6	2/11	2/19	4	2/24	10	
	5		8	2/11	2/17	6	2/27	10	9.8
#18	6	6.3	1	2/11	2/13	1	2/24	11	
	7		2	2/11	2/13	2	2/27	14	
	8		4	2/11	2/17	2	2/20	3	
	9		6	2/11	2/14	1	2/27	13	
	10		8	2/12	2/17	7	3/ 3	14	11.0
	11	6.0	1	2/10	2/13	3	2/20	7	
	12		2	2/10	2/13	2	2/20	7	
	13		4	2/11	2/13	1	2/20	7	
	14		6	2/11	2/17	7	3/ 3	14	
	15		8	2/12	2/14	2	3/ 3	17	10.4
	16	5.0	1	2/10	2/13	1	2/24	11	
	17		2	2/10	2/13	5	2/20	7	
	18		4	2/10	2/14	1	2/27	13	
	19		6	2/11	2/17	7	2/24	7	
	20		8	2/11	2/17	6	2/20	3	8.2
	21	4.0	1	2/10	2/13	4	2/20	7	
	22		2	2/10	2/13	7	2/18	5	
	23		4	2/10	2/14	2	2/24	10	
	24		6	2/11	2/14	2	2/27	13	
	25		8	2/11	2/14	2	2/27	13	9.6
	26	3.0	1	2/10	2/17	6	2/24	7	
	27		2	2/10	2/17	3	2/24	7	
	28		4	2/10	2/13	2	2/24	11	
	29		6	2/12	2/17	1	2/26	9	
	30		8	2/11	2/13	1	3/ 3	18	10.4

A duplicate of the experiment just described was carried on by adding to each vial a culture of yeast which is associated with D. brevicomis. The results are summed up in Table III.

Table III

Nutrient	Vial No.	pH	Drying Time	Date Eggs In	Date First Larvae	No. of First Larvae	Date of Last Larvae	Days	Average
	31	6.8	1	2/15	2/18	4	2/27	9	
H_2O	32		2	2/19	2/20	2	3/ 3	11	
	33		4	2/17	2/18	2	3/ 3	13	11.0
	34	7.0	1	2/15	2/18	2	2/27	9	
#18	35		2	2/19	2/20	3	3/ 3	11	
and	36		4	2/17	2/19	2	2/27	8	
Yeast	37		6	2/18	2/24	3	2/27	3	
	38		8	2/19	2/24	3	2/27	3	6.8
	39	6.0	1	2/15	2/19	1	2/27	8	
	40		2	2/19	2/24	5	3/ 3	7	
	41		4	2/17	2/19	1	3/ 7	16	
	42		6	2/18	2/24	3	3/ 3	7	
	43		8	2/18	2/24	6	3/ 7	11	9.8
	44	5.0	1	2/17	2/19	3	2/24	5	
	45		2	2/19	2/20	1	3/ 7	15	
	46		4	2/18	2/24	6	3/ 7	11	
	47		6	2/18	2/19	1	2/27	8	
	48		8	2/18	2/20	1	3/ 3	11	10.0
	49	4.0	1	2/17	2/20	4	2/24	7	
	50		2	2/18	2/19	1	3/ 3	12	
	51		4	2/18	2/19	1	3/ 3	12	
	52		6	2/18	2/24	7	3/ 3	7	
	53		8	2/18	2/20	2	2/24	4	8.4

The data shown in Tables II and III indicate that the larvae were able to survive over an average of about 9 days. No appreciable difference was observed by the addition of yeasts to the media, as indicated in Table III. The results of both experiments tend to show that petri dishes were more satisfactory than shell vials, since the average survival is apparently longer. This is explained, however, by the difference in recording data. Only that time between the date of first larvae and date of last death is recorded in Tables II and III, whereas the period between the date of planting and hatching of eggs is included in the total time in Table I. Since the eggs hatched from 1 to 5 days after planting, this time subtracted from the average for petri dishes would make the average about the same as for shell vials. If this is true, some factor beside the effect of fungi may prevent the further development of larvae. They were evidently not nourished, as the larvae which had no supply of food other than water survived just as long. The environment, therefore, was given greater consideration than heretofore.

Other Media Used

As a result of the above conclusions it was felt that different media should be used in order to obtain more natural conditions. The following substances were selected: ground inner bark of yellow pine, elder pith and boiled rice. Nutrients were added to elder pith and rice but not to the ground inner bark, as this was to be used as a check and should have all the natural food substances. Unfortunately, however, the eggs used were evidently infertile, as very few of them hatched. Those that did hatch were too few in number to warrant any positive conclusions.

Some of these substances were tried again during May, 1930, in order to test their utility, with a new supply of eggs. Ground inner bark, ground inner and outer bark, ground outer bark, agar-agar shreds and pith were used. Nutrient solution #18 was added to agar-agar shreds and pith, using pH values of 6, 5 and 4. Only enough nutrient solution was added to the agar-agar shreds and pith to make them fairly moist when tamped down. These media were then sterilized by the double-boiler method, i.e., by setting the containers in boiling water for 45 minutes.